Installation Guide

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English

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Before going into further details of the procedure for the instrument setup, we should give a simple explanation on how the instrument "reads" the samples to determine the ESR value.

The basic principle is the tube scanning made by the instrument through a light beam. The light intensity is such to have a maximum signal on the top of the tube (light passes through plastic and label), while blood shields the light beam, so that the signal level drops to minimum levels, then increasing again when the sensor reaches the bottom of the tube. The instrument draws a graph of light absorption as the one shown in Figure 1.

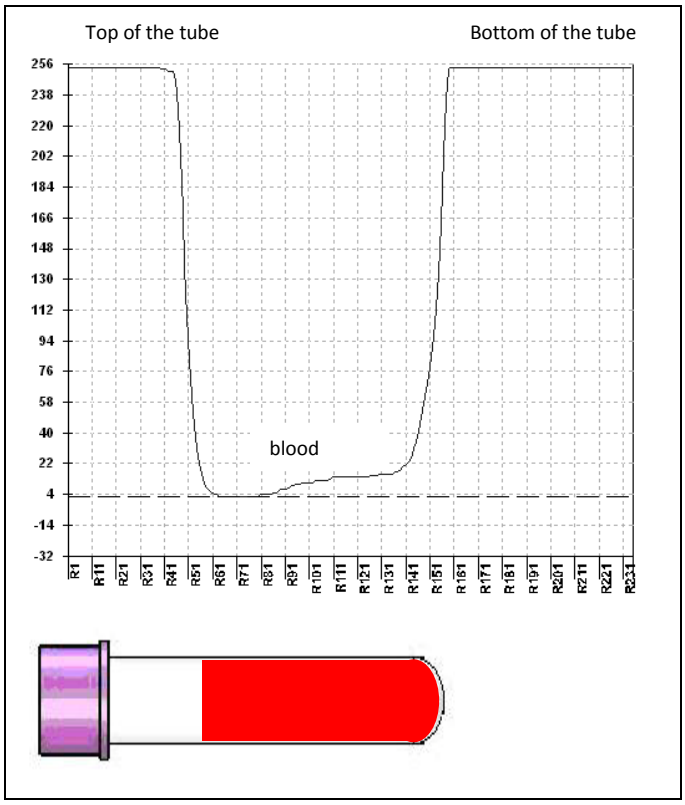
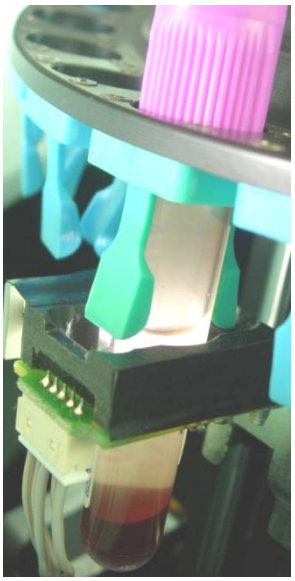


Figure 1: Graph of blood level reading inside the top lavender tube. The sensor moves up and down its shaft through a stepper motor. The instrument records both the number of steps run by the motor during the tube scanning and the intensity of light passing through the tube, from the top down to the bottom of the tube.



The ESR value is calculated by comparing the graph obtained at time zero (after sample mixing) with the one obtained after 20 minutes of sedimentation. The intensity of light, in fact, will pass through the plasma without any appreciable absorption (see Figure 2).

Time zero reading allows to determine the height of the blood column before sedimentation.

To determine the point of the graph corresponding to this height, the instrument uses two independent mathematical methods: M1 and M2.

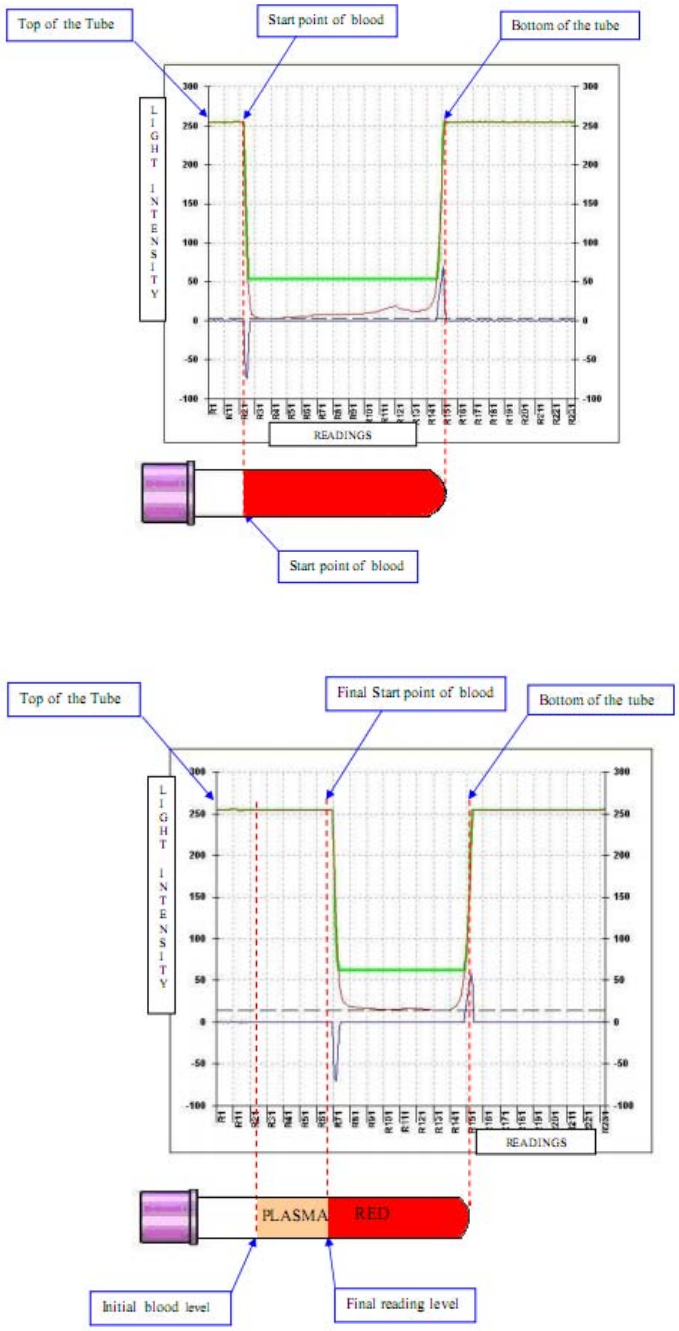


Figure 2: examples of graphs at time 0 and after 20 minutes of sedimentation. The graphs after processing with M1 filter (green) and after processing with M2 method (blue) are reported.

M1 method: starting from the original curve (in red in Figure 2), the graph is redrawn to make its analysis easier. To do this, it is necessary to apply a so-called "filter", that is merely a mathematical process that eliminates the noise from the original reading in the lower part (maximum light absorption) of the graph. In other words, the minimum value of light absorption is recalculated, by raising it in comparison to the real one, thus eliminating the background noise (green graph in Figure 2). Then, the point beyond which were recorded 4 consecutive increasing readings is searched by moving from right to left along the green graph. Basically, it is searched from which point light intensity increases, corresponding to the air/blood interface. The same method, applied to the graph obtained after sedimentation, allows to identify the point corresponding to the RBC/plasma interface (plasma does not appreciably absorb light). The difference between these two points is used to calculate the ESR value.

M2 Method: in this case, the graph derivative is calculated. The derivative changes its sign in the points where the graph changes slope (blue graph in Figure 2): the leftward point of changing slope corresponds to the air/blood interface in the case of the first reading, and to the RBC/plasma interface in the case of the reading after sedimentation. Again, the difference between these two points is used to calculate ESR value.

The software compares ESR values obtained by the two different methods and if they do not differ by more than 20 points, it provides the average of these two values as the result, otherwise it provides a “starred” result (M1).

Now we can start the description of the instrument setting procedure.
The two main parameters to be checked and modified, if needed, are:

- **light intensity (Duty Cycle PWM [%])**
- **percentage of filter (PERCFILTERMINABS)**

these parameters can be accessed from the service menu.

Since in the laboratories are used different test-tubes, blood volumes and labels, it is necessary to "customize" the instrument to fit to the drawing and sample management system of each laboratory.
First of all it is necessary to have some labeled tubes, containing samples from the routine, to observe their characteristics (brand, sample volume, type of labeling).

In this regards, the table below reports the minimum and maximum volume limits of the most common types of top lavender tubes which can be found on the market.

TUBE	MAX VOLUME	MIN VOLUME
VACUTAINER BD	4 ml	1.5 ml
VACUETTE GREINER	4.4 ml	2 ml
SARSTEDT	2.8 ml	1.5 ml
RUBBER	4.5 ml	2 ml

However, it should be considered that in private laboratories can be found unusual types of top lavender tubes, so mainly in these cases it is necessary to perform very accurate tests.

Regarding the labeling of tubes, refer to paragraph 4.2 (PREPARATION OF SAMPLES) of the USER MANUAL. The VES-Matic Cube 30 is designed to work with a maximum of 2 labels, applied to the sample to be tested, reading through a maximum of 3 layers of paper along the reading axis. It would be preferable to overlap the sample bar code label to the original one of the top lavender tube, so that the "back" of the tube is free. Check if it is possible to talk with the nurses who draw blood to suggest this method of labeling. *This is indeed the way of labeling that causes the fewest problems and also allows a visual assessment of blood volume in the tube and of the sedimentation level of the sample*.

It is very important the **proper positioning of the tube in the rotor**: labels must face the LED (see Figure 3). In this way the quota of light reaching the samples will be the same for all.

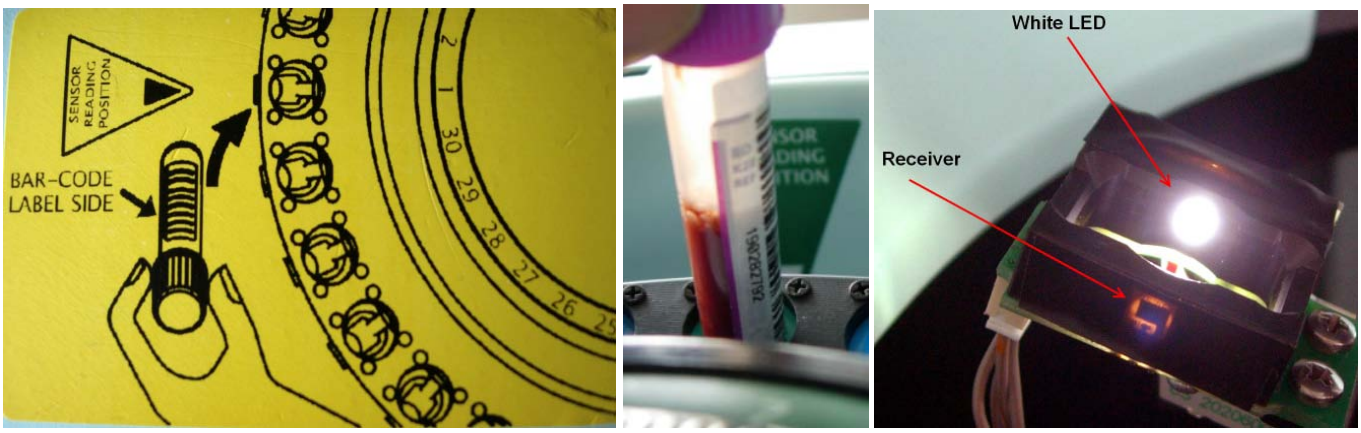


Figure 3: from left to right 1. label explaining the correct insertion of the tubes, applied on the chassis; 2. Tube position 3. reading sensor: the labels must face the LED.

Using the laboratory tubes, the first step to be performed is the calibration of light intensity;
the default value of setting of light intensity is 20. To change parameters:

Enter the set-up → Service (password = 000000) → Motor test → reading unit test → reading test → **PWM duty cycle (%)**

Insert one of the tubes (after thorough mixing) in position 1 of the rotor and perform an initial scan with the default light value and check the reading graph (to perform this operation the lid must be closed). If you get a reading curve like the one in Figure 1, with no spikes anywhere, the default value can be kept. This test should be run on 3-4 different samples, verifying that the reading curve is "clean" for all samples.

In case of abnormal curves, you must modify the intensity of light, trying to get a "clean" graph, see Figure 4.

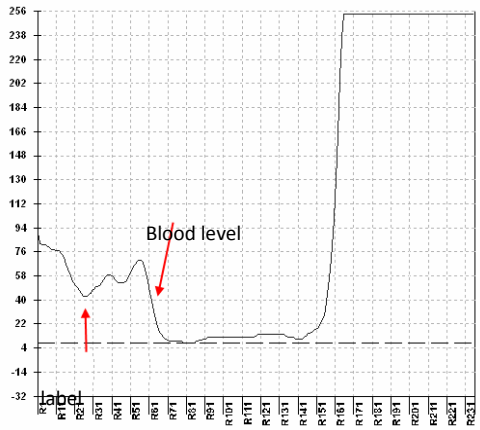
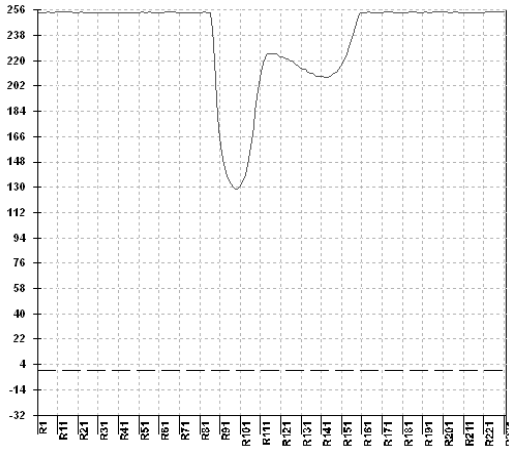
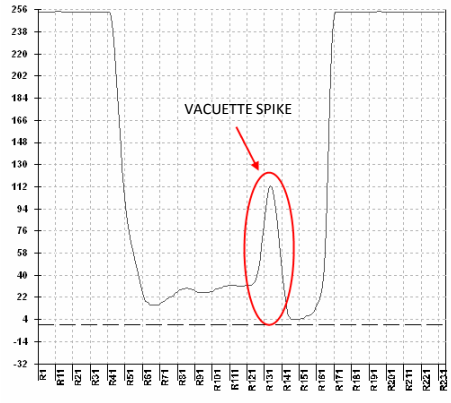
The default level of light intensity in this case is <u>too low</u>: the labels shield the light and do not allow a clear distinction of the air/blood interface. By increasing the light intensity it is possible to reduce the influence of the label: infact the label, or labels, will be crossed by the light beam, while blood will not).	
The default level of light intensity in this case is <u>too high:</u> light reaches the receiver by "sliding" along the walls of the tube, making it "blind". This may happen in the case of little-known brands of top lavender tubes manufactured with cheaper plastic materials (often the walls of these tubes are opalescent, and this could explain the phenomenon). In this case, you should decrease the intensity of light in order to alleviate the "sliding" effect. In these situations it is advisable to convince the laboratory of the necessity of a proper labeling, so that the labels have the minimum possible influence on the absorption of light.	
The default level of light intensity in this case is too high: this is the typical situation which happens with Greiner VACUETTE, with the generation of a spike of light at the bottom of the tube. By lowering the intensity of light the phenomenon may be eliminated.	

Figure 4: Examples of "abnormal" graphs

Note *the value of light which is set on the instrument is the one of the last performed reading test.*

Graph analysis

In the **Graphs** menu (set up → support (password 000000) → Graphs) it will be possible to observe the graphs of the samples tested in the last cycle of analysis.

By pressing in sequence the "Graph" key you can view the graphs related to:

- First (or reference) reading (Time 0)
- Second reading (Time = 20 min)
- First and second reading

for each sample.

To move from one sample to the next, press the arrow keys; for each sample it is displayed the corresponding position in the sample holder plate (not the patient bar code).

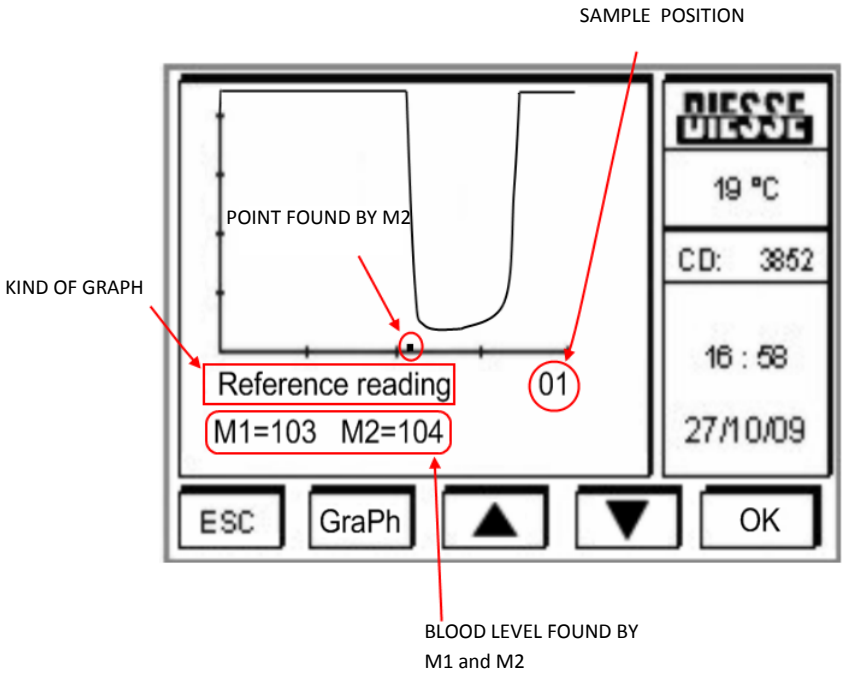


Figure 5: Graph display first reading

In the *"first"* and *"second"* reading graphs (see Figure 5) are shown the blood levels found with the two main calculation methods (M1 and M2) in addition to the graph related to the specific reading.

Below the graph it is shown the numeric value related to blood height, expressed in number of steps (the higher is the number, the lower will be the level of blood) which is also displayed in the graph:

■ represents the point (related to the x axis) where M2 method found blood level

□ represents the point where M1 method found blood level (in the above reported figure, this symbol can not be seen as M1 and M2 are identical so it is not possible to distinguish them).

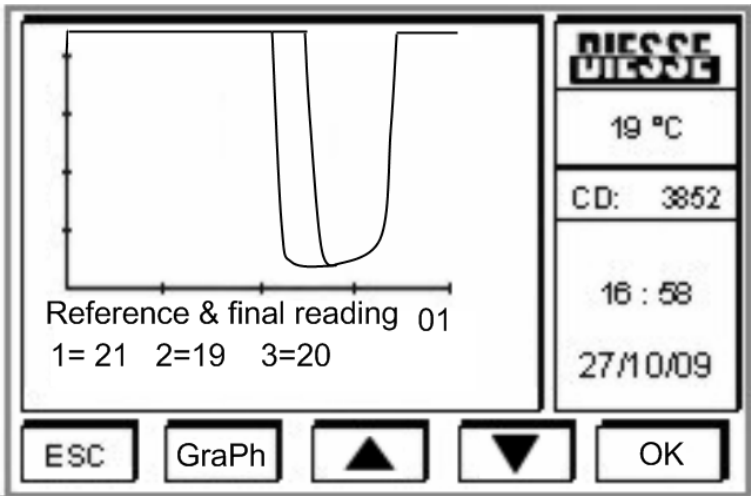


Figure 6: Overlapping graphs of first and second reading

In the "*first and second reading*" graph, which shows the overlapping graphs, instead of the number representing the blood levels of single readings found by M1 and M2, is shown the difference in mm/h (sedimentation) between the levels of the two readings found by various methods (Figure 6).

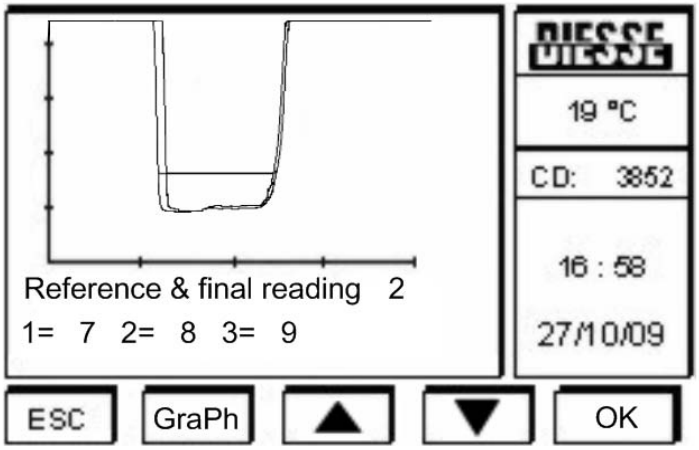
Check of M1 filter

In Figure 7 there are two examples, one of "clean" curves and another with problematic curves due to a reading spike (VACUETTE Greiner type). In the first case, there is no need to change any parameters, because the instrument read the level of sedimentation without problems. In the second case, where the true ESR value was 22, the M2 method correctly calculated ESR, while the M1 method, because of the spike, gave an incorrect result. By raising the M1 filter level (PERCFILTERMINABS: Percentage value that controls the filter height in Method 1. Accessible through Service – Set/Change values entry) the base line of the graph raises.

Clean curve

No parameters to be changed

2	REF	READ	DIFF	ESR
M1	45	52	7	7
M2	45	53	8	8



Problematic curve

In this case the M1 filter should be raised to prevent M1 being influenced by the spike

1	REF	READ	DIFF	ESR
M1	71	75	4	4
M2	64	80	16	22
M3	78	80	2	2

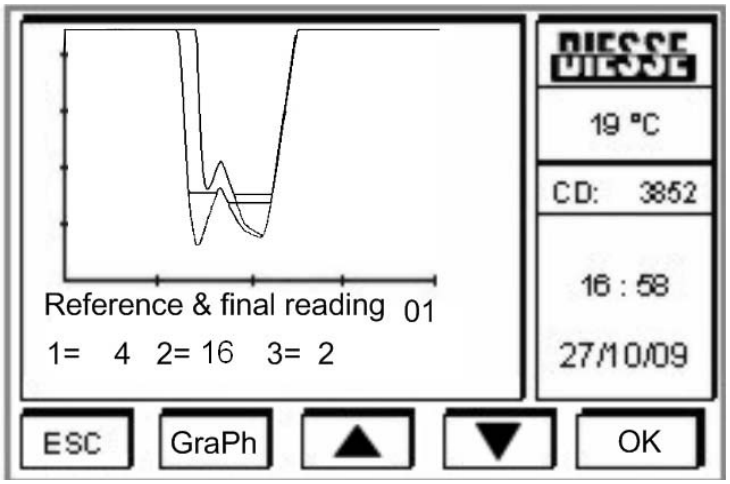


Figure 7: graph examples